

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s) : SLIJKHUIS ET AL.
Serial No. :
Filed : Concurrently Herewith
For : PROCESS FOR OXIDATION OF STEROIDS AND
GENETICALLY ENGINEERING CELLS USED THEREIN
Group Art Unit : 1652
Examiner : D. Steadman

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

This amendment is submitted in response to the Notice to Comply with the Sequence Rules mailed September 3, 1998. The Notice requested that a substitute Sequence Listing be presented as the computer readable form of the Sequence Listing filed June 17, 1998 was found to be damaged and/or unreadable.

Accordingly, this amendment includes a paper copy of the substitute Sequence Listing (Exhibit 1), a diskette containing the substitute Sequence Listing in computer readable form (Exhibit 2), and a statement under 37 C.F.R. §1.825(b) or §1.825(d) (Exhibit 3) that the substitute paper copy of the Sequence Listing and the substitute computer readable Sequence Listing are identical.

Please replace the Sequence Listing filed June 17, 1998 with the enclosed substitute paper Sequence Listing and insert the substitute Sequence Listing after page 98 of the specification of the application and before the claims.

As this amendment does not introduce any new matter into the application, entry of the amendment and of the paper copy of the substitute Sequence Listing are respectfully requested.

Respectfully submitted,
BIERMAN, MUSERLIAN AND LUCAS, L.L.P.

Dated: February 26, 2002

By 
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Our Ref.: 146.1169-CON-1-DIV-1-CON

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: : Examiner: D. Steadman
SLIJKHUIS et al :
Serial No.: : Group: 1652
Filed: Concurrently Herewith :
For: PROCESS FOR...USED THEREIN :
: 600 Third Avenue
: New York, NY 10016
: February 22, 2002

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Please amend this application as follows:

IN THE SPECIFICATION:

This application is a Continuation of U.S. Patent Application Serial No. 09/098,990 filed June 17, 1998, now abandoned which is a division of U.S. Application Serial No. 418,085 filed April 6, 1995, now U.S. Patent No. 5,869,283, which is a continuation-in-part of U.S. Patent Application Serial No. 054,185 filed April 26, 1993 which is a continuation of U.S. Patent Application Serial No. 474,857 filed October 30, 1990, now abandoned and U.S. Patent Application Serial No. 002,608 filed January 11, 1993 which is a continuation of U.S. Patent Application Serial No. 474,798 filed July 16, 1990, now abandoned.--

Please add the following new claims:

--34. An expression cassette comprising a heterologous DNA encoding two or more bovine or human enzymes selected from the group consisting of SCC, ADR, ADX, 3β -HSD, steroid 17 α -hydroxylase, NADPH, RED, steroid 21-hydroxylase and steroid 11 β -hydroxylase from the metabolic pathway for the bioconversion of cholesterol to hydrocortisone wherein one of the enzymes catalyzes the oxidation of cholesterol to pregnenolone with a bovine enzyme and the remaining one or more enzymes catalyze at least one reaction selected from the group consisting of: the oxidation of pregnenolone to progesterone; the oxidation of progesterone to 17 α -hydroxyprogesterone; the oxidation of 17 α -hydroxyprogesterone to cortexolone; and the oxidation of cortexolone to hydrocortisone; and wherein the heterologous DNA is operably linked to control sequences required to express the encoded enzymes in a recombinant host.

35. An expression cassette comprising a heterologous DNA encoding a human and bovine enzyme selected from the group consisting of SCC, ADR, ADX, 3β -HSD, steroid 17 α -hydroxylase, NADPH, RED, steroid 21-hydroxylase and steroid 11 β -hydroxylase from the metabolic pathway for the bioconversion of cholesterol to hydrocortisone which enzyme catalyzes the oxidation of cholesterol to hydrocortisone which enzymes catalyzes the oxidation of cholesterol to pregnenolone and further comprising one or more

additional heterologous DNAs encoding one or more bovine or human additional enzymes from the metabolic pathway for the bioconversion of cholesterol to hydrocortisone, which one or more additional enzymes catalyze at least one reaction selected from the group consisting of: the oxidation of pregnenolone to progesterone; the oxidation of progesterone to 17 α -hydroxyprogesterone; the oxidation of 17 α -hydroxyprogesterone to cortexolone; and the oxidation of cortexolone to hydrocortisone; and wherein each of the heterologous DNAs is operably linked to control sequences required to express the encoded enzymes in a recombinant host.

36. The expression cassette according to claim 34 wherein the enzyme that catalyzes the oxidation of cholesterol to pregnenolone is side-chain cleaving enzyme ($P_{450}SCC$) and the remaining one or more enzymes are selected from the group of: 3 β -hydroxysteroid deshydrogenase/isomerase (3 β -HSD); adrenodoxin (ADX); adrenodoxin (ADR); steroid-17- α -hydroxylase ($P_{450}17\alpha$); NADPH cytochrome P_{450} reductase (RED); steroid-21-hydroxylase ($P_{450}C21$); and steroid-11- β -hydroxylase ($P_{450}11\beta$).

37. The expression cassette according to claim 36 characterized in that the heterologous DNA coding sequences originate from human or bovine species.

38. The expression cassette according to claim 36 wherein the enzyme that catalyzes the oxidation of cholesterol to pregnenolone

is side-chain cleaving enzyme ($P_{450}SCC$) and the remaining one enzyme is adrenodoxin (ADX).

39. The recombinant host cell and progeny thereof comprising at least one expression cassette according to claim 34.

40. The recombinant host cell and progeny thereof according to claim 39, wherein the host is a micro-organism.

41. The recombinant host cell and progeny thereof according to claim 40, wherein the host is a species of *Saccharomyces*, *Kluyveromyces* or *Bacillus* or *Escherichia coli*.

42. A process for making two or more bovine or human enzymes selected from the group consisting of SCC, ADR, ADX, 3β -HSD, steroid 17 α -hydroxylase, NADPH, RED, steroid 21-hydroxylase and steroid 11 β -hydroxylase from the metabolic pathway for the bioconversion of cholesterol to hydrocortisone comprising incubating the recombinant host cell of claim 41 in a nutrient medium under conditions where the two or more enzymes encoded by the heterologous DNA are expressed and accumulate.

43. A recombinant host cell and progeny thereof comprising a heterologous DNA encoding two or more bovine or human enzymes selected from the group consisting of SCC, ADR, ADX, 3β -HSD, steroid 17 α -hydroxylase, NADPH, RED, steroid 21-hydroxylase and

steroid 11 β -hydroxylase from the metabolic pathway for the bioconversion of cholesterol to hydrocortisone wherein one of the enzymes catalyzes the oxidation of cholesterol to pregnenolone and the remaining one or more enzymes catalyzes at least one reaction selected from the group consisting of: the oxidation of pregnenolone to progesterone; the oxidation of progesterone to 17 α -hydroxyprogesterone; the oxidation of 17 α -hydroxyprogesterone to cortexolone; and the oxidation of cortexolone to hydrocortisone, and wherein the heterologous DNA is operably linked to control sequences required to express the encoded enzymes in the recombinant host.

44. The recombinant host cell according to claim 43 wherein the enzyme that catalyzes the oxidation of cholesterol to pregnenolone is side-chain cleaving enzyme (P₄₃₀SCC) and the remaining one or more enzymes are selected from the group of: 3 β -hydroxysteroid deshydrogenase/isomerase (3 β -HSD); adrenodoxin (ADX); adrenodoxin reductase (ADR); steroid-17- α -hydroxylase (P₄₅₀17 α); NADPH cytochrome P₄₅₀ reductase (RED); steroid-21-hydroxylase (P₄₅₀C21); and steroid-11- β -hydroxylase (P₄₅₀11 β).

45. The recombinant host cell according to claim 47 wherein the enzyme that catalyzes the oxidation of cholesterol to pregnenolone is side-chain cleaving enzyme (P₄₅₀SCC) and the remaining one or more enzymes includes at least adrenodoxin (ADX).

46. The recombinant host cell of claim 47 wherein the host cell is *Kluyveromyces* species and wherein the enzyme that catalyzes the oxidation of cholesterol to pregnenolone is side-chain cleaving enzyme ($P_{450}SCC$) and the remaining one or more enzymes includes at least adrenodoxin (ADX).--

REMARKS

The amendment is presented to insert reference to the parent applications and their status and to present the claims as finally presented in application 09/098,990.

All of the claims in the last application were rejected under 35 USC 103 as being obvious over the Zuber et al reference taken in view of the Hoffman et al patent. The Examiner states that Zuber et al transforms eukaryotic cells containing expression vectors encoding P_{450} side chain cleaving enzyme and adrenodoxin and a method of producing P_{450} side chain cleaving enzyme and adrenodoxin and concedes that Zuber et al does not teach an expression vector encoding both P_{450} side chain cleaving enzyme and adrenodoxin but cites Hoffman et al as teaching the expression of two subunits of hemoglobin by expression of both proteins on one expression vector and that it teaches co-expressing proteins in Saccharomyces cerevisiae and concedes that it does not teach the expression vector but he deems that it would have been obvious to combine the references to obtain Applicants' results.

Applicants respectfully traverse this ground of rejection since one skilled in the art would not combine the references as the Examiner has done with the benefit of Applicants' teaching. It is deemed that the Hoffman et al patent is an improper reference for the present application since the present application is entitled to the filing dates of September 23, 1998, May 6, 1998 and September 25, 1989, all of which support the present claims.

The Zuber et al article makes an expression in the host which is a mammalian cell whose characteristic is to possess already endogenous adrenoxin and adrenoxin reductase even in small amounts as well as the cellular organization for encoding a mitochondrial processing (maturization) permitting the working of the natural sequences of P₄₅₀ SCC and ADX which are described in the article. The production of the reference is very low since the cells COS are not convenient for an industrial bioconversion process and the natural cDNA from P₄₅₀ SCC, ADX, and ADR do not work in a bacterial cell. In order to make Applicants' process work, the inventors had to use modified sequences (mature, SCC, ADR and ADX) and to introduce the entire enzymatic chain (SCC, ADR and ADX) to have the process work. One skilled in the art before Applicants' invention, would not have known whether or not there was a possibility of a functional expression of mitochondrial P₄₅₀ in a microorganism. Only the use of a bacterial host instead of a mammalian cell host permits productivities comparable with an industrial process and, therefore, the references do not teach Applicants' invention and

withdrawal of the same is requested.

In view of the amendments to the claims and to the above remarks, it is believed that the claims clearly point out Applicants' patentable contribution and favorable consideration of the application is requested.

Respectfully submitted,
BIERMAN, MUSERLIAN AND LUCAS



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Enclosures: Marked-Up Version of Specification
Return Receipt Postcard

MARKED-UP VERSION OF SPECIFICATION

PROCESS FOR OXIDATION OF STEROIDS AND

GENETICALLY ENGINEERED CELLS USED THEREIN

--See Insert A--

ABSTRACT OF THE DISCLOSURE

An expression cassette, operable in a recombinant host, comprising a heterologous DNA coding sequence encoding a protein, which is functional, alone or in cooperation with one or more additional proteins, of catalyzing an oxidation step in the biological pathway for conversion of cholesterol into hydrocortisone, which step is selected from the group consisting of:

- the conversion of cholesterol to pregnenolone;
- the conversion of pregnenolone to progesterone;
- the conversion of progesterone to 17 α -hydroxy-progesterone;
- the conversion of 17 α -hydroxyprogesterone to cortexolone;
- the conversion of cortexolone to hydrocortisone, and the corresponding control sequences effective in said host.

Insert A

This application is a Continuation of U.S. Patent Application Serial No. 09/098,990 filed June 17, 1998, now abandoned which is a division of U.S. Application Serial No. 418,085 filed April 6, 1995, now U.S. Patent No. 5,869,283, which is a continuation-in-part of U.S. Patent Application Serial No. 054,185 filed April 26, 1993 which is a continuation of U.S. Patent Application Serial No. 474,857 filed October 30, 1990, now abandoned and U.S. Patent Application Serial No. 002,608 filed January 11, 1993 which is a continuation of U.S. Patent Application Serial No. 474,798 filed July 16, 1990, now abandoned.--